412–418 Nucleic Acids Research, 1994, Vol. 22, No. 3

Analysis of *FAS3/ACC* regulatory region of *Saccharomyces cerevisiae*: identification of a functional UAS_{INO} and sequences responsible for fatty acid mediated repression

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Received September 17, 1993; Revised and Accepted December 21, 1993 GenBank accession no. M92156

ABSTRACT

We have determined the sequence of the *FAS3/ACC* regulatory region and mapped the transcription initiation site. In this sequence, there are two putative UAS_{INO} sequences. Deletion and mutational analyses revealed that the UAS_{INO} sequence at nucleotides −719 to −710 is functional. The expression of *FAS3-lacZ* reporter genes and the measurement of mRNA levels in regulatory mutants of phospholipid biosynthesis clearly indicated that *FAS3* is regulated by inositol and choline. Previous studies have shown that the genes coding for fatty acid synthase, *FAS1* and *FAS2*, are regulated by inositol (Chirala, S.S. [1992] *Proc. Natl. Acad. Sci. USA* 89, 10232–10236). Thus all three genes involved in saturated fatty acid biosynthesis are coordinately regulated with phospholipid biosynthesis. Comparison of the UAS_{INO} sequences present in *FAS1*, *FAS2*, and *FAS3* suggested that the functional sequence of this UAS element is YTTCACATG. However, even when the functional UAS_{INO} was mutated, substantial expression of the *FAS3-lacZ* reporter gene was observed. Deletion analysis, electrophoretic mobility shift assays, and expression using a heterologous reporter gene showed that the region between nucleotides −840 and −736 has two UAS elements. The same sequence seems to be responsible for fatty acid-mediated repression of *FAS3*. The presence of these additional UAS sequences explains why yeast does not require fatty acids even when repressing amounts of inositol and choline are present in the medium.

INTRODUCTION

Fatty acid synthesis in the yeast Saccharomyces cerevisiae involves three structural genes. *FAS1 and FAS2* respectively code for the β and α subunits of fatty acid synthase (FAS) and *FAS3/ACC* codes for acetyl-CoA carboxylase (ACC). ACC catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, which is the committed step in fatty acid synthesis. FAS catalyzes the synthesis of lon-chain fatty acids from acetyl-CoA and malonyl-CoA in the presence of NADPH. In yeast, FAS consists of the two multifunctional subunits (α and b) that are organized in an $\alpha_6\beta_6$ complex (1). The three *FAS* genes have been cloned and sequenced (2–8). Numa and coworkers have shown that acetyl-CoA carboxylase is repressed by fatty acids (9, 10). We recently showed that all three genes are coordinately repressed by fatty acids and that the activities of FAS and ACC are reduced in the presence of inositol (11). We and other investigators have shown previously that the regulation of *FAS1* and *FAS2* by inositol and choline is mediated through UAS_{INO}/UAS_{ICRE} present in these genes (11, 12). UAS_{INO} was originally identified as a conserved nonamer sequence present in several genes involved in phospholipid biosynthesis (11–14). However, the functional sequence, TYTTCACATG, is a decamer (11, 12). Since the three genes are coordinately regulated (11) it is necessary to sequence the regulatory region of *FAS3* and perform deletion and mutational analysis of the region to determine the *cis*-acting elements that regulate this gene. Here we report the identification of two new UAS sequences that seem to play a role in fatty acid mediated repression. In addition the presence of a functional UAS_{INO} in the regulatory region of *FAS3/ACC* clearly indicates that this UAS is needed for coordinated regulation of the genes involved in lipid biosynthesis.

MATERIALS AND METHODS

Yeast strains

In most of these experiments we used the *S.cerevisiae* strain SEY2102 (*MATα*, his3, leu2, *ura3*). Regulatory mutants, it8 (*MATa, ino2-21, ura3-1, lys2*), SAH1028 (*MATa, ino4, leu2-3, 112, ura3-1*), and SAH1032 (*MATα*, ino4, ino2-21, ura3-1) of *INO1* gene and the isogenic wild-type W303-1A (*MATa, ade2-1, can1-100, his3-11,15, leu2-3, 112, trp1-1, ura3-1*) were obtained from Dr Susan Henry, Carnegie Mellon University, Pittsburgh. The yeast strain BJ5465 (*MATa, ura3-52, trp1, leu2, leu2Δ1,*

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his3Δ200, pep4::His3, prb1 Δ1.6R, can1, GAL) was obtained from the Yeast Genetic Stock Center (Berkely, California).

Media and growth conditions

Synthetic medium prepared from yeast nitrogen base (DIFCO), which contains about 11 μM inositol, or a medium prepared from salts and vitamins based on the same composition but lacking inositol (−inositol medium) was used with appropriate nutritional supplements. Glucose was the carbon source for all the experiments. The fatty acid medium contained 2 mM myristic acid and 0.5% Tween 40. The concentrations of the phospholipid precursors used are given in the legends to the figures and tables. For the electrophoretic mobility shift assays, yeast strain BJ5465 was grown in a medium containing 1% yeast extract, 2% peptone, and 2% dextrose (YPD). All experiments were performed with mid-log phase cells grown to an optical density of 0.4 to 0.8 at 600 nm.

Sequence determination and mapping of the transcription initiation site

A *SalI*−*Hind*III fragment containing the regulatory region of *FAS3* was subcloned from EMBL-ACC in pUC18 (FAS3-Sal) and sequenced by using standard procedures as described (6, 8). A synthetic primer (40 bp) complementary to the *FAS3* sequence from nucleotides +5 to +45 was end-labeled by using [γ-^{32}P]-ATP and polynucleotide kinase (17). Primer extension analysis employing reverse transcriptase, yeast total RNA and the [^{32}P]-labeled oligonucleotide was performed according to standard protocols as described (17, 18).

Construction of the *lacZ* reporter gene and determination of β-galactosidase activity

The 242 bp fragment (−226 to +16) *Eco* RI−*Hind* III fragment was cloned into pSCFAS1-lacZ reporter plasmid (a *CEN4, ARS* plasmid with *URA3* as a selectable marker [11]) by replacing the *FAS1* regulatory region. For this cloning, the plasmid pSCFAS1-lacZ was cut with *Bam*HI, filled in, and cut with *EcoR I* and used as vector. The 242 bp *Eco*RI−*Hind*III fragment blunt ended at the *Hind* III site was cloned into this vector. The resulting construct had an in-frame translation stop codon that was generated by blunt-end ligation of the *Hind* III and *Bam*HI sites (GAA AGC TGA TCC). Using PCR methods, the stop codon TGA was changed to GGA. This change introduced a *Bam*HI site. The recloning of this PCR fragment into pSCFAS1-lacZ at *Eco*RI and *Bam*HI sites produced the plasmid called pFAS3-Eco. The *Eco*RI fragment from the pFAS3-Sal, containing the *SalI*−*Eco*RI region (nucleotides −169 to −227; the upstream *Eco*RI in this fragment is from the multiple cloning sites of pUC18) was cloned into the unique *Eco*RI site of pFAS3-Eco in the proper orientation. For PCR-mediated deletions we used primers that were 20 nucleotides long with *Eco*RI recognition sites. To make the 5′ deletions, we used the 5′-specific primers from the sequence of the *FAS3* regulatory region and a 3′ common primer from the sequence of the *lacZ* reporter gene (19, 20). To generate internal fragments to be tested for regulatory function, both primers were derived from the *FAS3* regulatory region. All the fragments generated by PCR were cut with *Eco*RI and cloned into pFAS3-Eco or into *CYC1-lacZ* reporter gene vectors. The orientation of the fragments was checked by sequencing with appropriate primers. The β-galactosidase activity was determined in permiabilized yeast cells (21) using o-nitrophenyl-β-D galactoside as substrate. The cell density was measured at 600 nm and the o-nitrophenol formed at 420 nm. The specific activity was calculated using the formula $(OD_{420}\times 1000)/(OD_{600}\times t\times v)$ and expressed as units/min/OD_{600}. The specific activity of β-galactosidase determined from three independent transformants of the same construct varied less than 5%. The same construct when retransformed and assayed the variation was about 10 to 15%. To normalize for this variation we have included either the construct containing the entire regulatory region or one of the already examined deletion constructs while testing new deletion constructs as they became available. The values obtained with the known deletion constructs were averaged and the results obtained with the new deletions were normalized.

Miscellaneous procedures

Yeast transformations (22), RNA isolation (4), northern analysis (11, 23), site-directed mutagenesis (11, 24), DNA sequencing (4, 6, 8), and gel retardation analysis (11, 25) were performed as described.

RESULTS

Regulatory region of the *FAS3*

The sequence of the *FAS3* regulatory region is shown in Figure 1. The transcription initiation site as determined by primer extension analysis is between nucleotides −16 and −9. The S1 mapping analysis did not reveal any other upstream initiation sites (data not shown). There are putative ABF1, GRF1, REB1, and UAS_{INO} sequences (11, 12, 26, 27) as indicated in Figure 1. In addition, there are unique sequences, such as (CAAGAA)5, starting at nucleotide −910 and an overlapping run of (CGAYAC)7 at nucleotide −145. The decamer (GGCCAAAAAC) and nonamer sequence (AGCCAAGCA) conserved in *FAS1* and *FAS2* (11) are not found in this sequence. TCATTATG, a sequence conserved between *FAS1* and *FAS2* that encompasses the Met codon (6), is also not found in the *FAS3* sequence. The latter three sequences might be important in maintaining the subunit stiochiometry of the α and b subunits of FAS.

Deletion analysis of the *FAS3* regulatory region

The construction of *FAS3-lacZ* reporter gene with basal level expression (pFAS3-Eco) is described in Materials and Methods. The 5′ deletions were generated by either using available restriction sites or by using PCR methods as described in Materials and Methods. These deletions were analyzed as *lacZ* reporter gene constructs, and the results are summarized in Figure 2. Based on the 5′ deletion analysis, the role of the putative regulatory sequences ABF1, GRF1, and REB1 is not clear. However, the region between nucleotides −976 to −693, represented by pFAS3-Sst and pFAS3-Bst in Figure 2A, is critical for the expression of this gene. Deletion of this region led to about a 10-fold reduction in the expression levels of *lacZ* (Figure 2A). This region contains the UAS_{INO} and the unique (CAAGAA)5 and CACATCTCTC sequences (Figure 1). Further deletions in this region showed that the unique (CAAGAA)5, and CACATCTCTC sequences, did not affect the expression of the reporter gene under the growth conditions used. However, when the region between nucleotides −866 and −744 was deleted, expression of the reporter gene was reduced by about 60%. These results suggested that the region between −866 and −744 contains some activator sequences (see below). Fatty acid-mediated repression is reduced when the sequence between −840

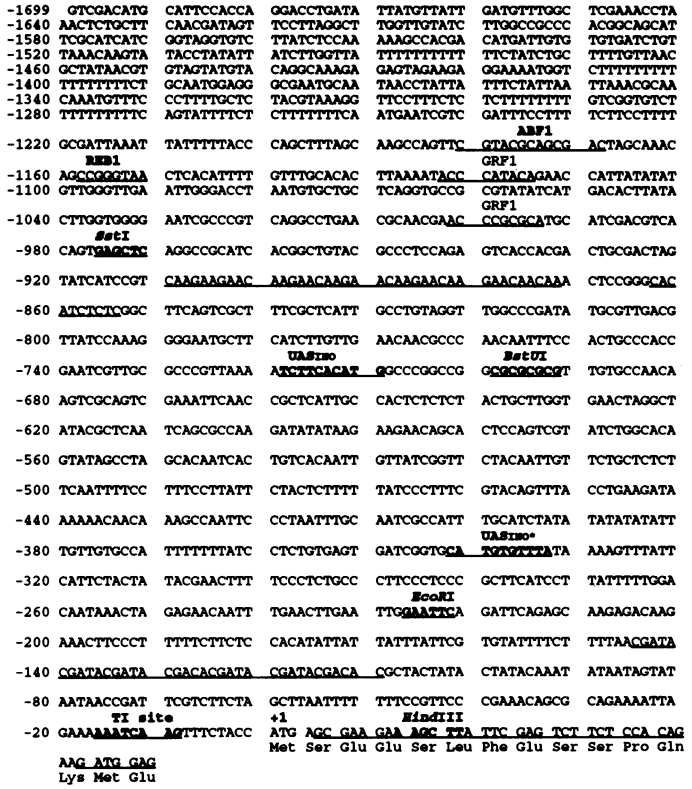

Figure 1. Nucleotide sequence of the *FAS3* regulatory region. The sequence begins with a *Sal*I site, and numbering begins with ATG codon. The restriction sites used in making the deletions, the unique sequences such as the GAACAA runs, the putative UAS sequences, the transcription initiation site, and the primer used to map the transcription initiation site (which is complementary to the sequence at +5 to +44) are indicated by underlining. UAS_{INO*} denotes similar but not quite well-conserved UAS_{INO} sequence.

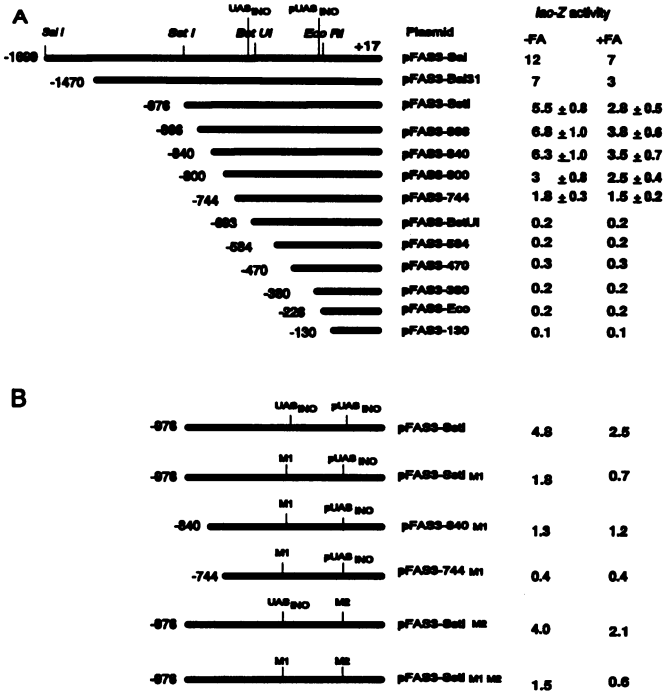

Figure 2. Deletion and mutational analyses of the *FAS3* regulatory region. (**A**). 5′ deletions of the regulatory region; all deletions were constructed using pFAS3-Eco (Figure 3). Available restriction sites and PCR methods were used in cloning these deletions into the reporter gene vectors as described in 'Materials and Methods'. The β-galactosidase activity was determined in whole cells. The average of the values obtained from three independent trasnformants for each plasmid are reported as described in 'Materials and Methods'. The −FA and +FA refer to absence or presence of fatty acids in the medium, respectively. Some of the critical deletions were reassayed and the standard deviation values calculated for them are also reported. (**B**). Mutational analysis of UAS_{INO} sequences in *FAS3*. The *Sst*I−*Bam*HI fragment from pFAS3-Sst was cloned in M13 vector and mutagenesis was performed according to Knuckle's method as described (11, 24). The sequence from −716 to −707 was converted from T-CTTCACATG to TCTTCCCGGG (M1), and the sequence from −343 to −333 was converted from GCATGTGTTTA to GGATCCGTTA (M2). The mutated fragments were cloned as *Eco*RI fragments in pFAS3-Eco and the *lacZ* activity determined as described above. The PCR-mediated deletions were made to generate the −840 and −740 subclones.

and −744 is deleted. When the sequence between −744 and −693 is deleted expression of the reporter gene is severely reduced . One of the putative UAS_{INO} sequences is present in this region (Figures 1 and 2A). Deletions toward the Met codon from −693 did not result in any further changes in the expression of the reporter gene. In addition to the UAS_{INO} sequence at −719 to −710, there is another putative UAS_{INO*} sequence at −342 to −333. Based on the deletion analysis shown in Figure 2A, the UAS_{INO*} at −342 apparently is nonfunctional. Similarly, the (CGAYAC)7 sequence at −140 is seemingly nonfunctional under the growth conditions used in these experiments.

Mutational analysis of the UAS_{INO} sequences in the *FAS3* regulatory region

Results from the deletion analysis described above suggested that the UAS_{INO} sequence located at nucleotides −719 to −710 is functional. To further confirm these results, we performed site-directed mutagenesis of these UAS_{INO} sequences and tested the mutant constructs in reporter gene expression. We have converted the putative UAS_{INO} sequence at −719 to −710 from TCTT-CACATG to TCTTCCCGGG (M1) and the sequence at −342 to −333 from GCATGTGTTTA to GGATCCGTTA (M2). These mutations generated *Sma*I and *Bam*HI sites respectively, which facilitated confirmation of successful mutagenesis. As shown in Figure 2B, mutating the UAS_{INO} sequence at −719 resulted in about 60% loss in the expression of the pFAS3-lacZ reporter gene. However, based on the mutational and deletion analyses the putative UAS_{INO*} sequence at −342 is nonfunctional (Figure 2A and B). The complementary sequence TAAACACATG of this putative UAS_{INO*}, contains only six nucleotides of the consensus TYTTCACATG, suggesting that the stretch of four pyrimidines at beginning of this motif is important for UAS function. The mutational analysis confirms that TCTTCACATG sequence at −719 is a functional UAS_{INO}. Mutation of the UAS_{INO} sequence at −719 in pFAS3-Sst (M1), did not completely abolish the expression of *lacZ* reporter gene (Figure 2B). In addition, deletion of the region between −840 and −744 in M1 mutant results in very low level expression of the reporter gene. These results suggest that there are additional UAS sequences in this region. The data shown in Figure 2A and B, were obtained with cells grown in synthetic dextrose medium containing 11 mM inositol. The reduction in expression of the reporter gene under these repressing conditions when the UAS_{INO} was mutated suggests that the UAS_{INO} is a positive regulator of *FAS3* as in *FAS1* and *FAS2* (11).

Table 1. Analysis of sequences responsible for fatty acid-mediated repression

Regulatory sequence	Vector	*lacZ* activity -FA	*lacZ* activity +FA
None	pFAS3-Eco	0.3	0.3
GRF1	*CYC1-lacZ*	4.5	5.0
	pFAS3-Eco	1.0	0.9
UAS_{INO}	*CYC1-lacZ*	17.0	17.0
FAS3 -866 to -648	*CYC1-lacZ*	30.0	16.0
(contains UAS_{INO})	pFAS3-Eco	2.0	1.2
FAS3 -840 to -648	*CYC1-lacZ*	27.0	16.0
(contains UAS_{INO})	pFAS3-Eco	2.0	1.3
FAS3 -840 to -736	*CYC1-lacZ*	7.8	5.5
FAS3 -800 to -736	*CYC1-lacZ*	2.6	1.9

As shown Figure 2A, the sequence between −840 and −744 seems to play a role in fatty acid-mediated repression. These sequences were tested using homologous pFAS3-Eco and heterologous *CYC1-lacZ* reporter genes. The *lacZ* reporter gene assays were performed as described in the legends to Figure 2. The GRF1 sequence used here is AAACAAAACCCAGACATCAT (26) and the UAS_{INO} sequence is TCTTCACATG. Both nucleotides were double-stranded and had *Eco*RI recognition sites at the ends to facilitate cloning.

Table 2. Phospholipid precursor-mediated repression of the *FAS3* reporter gene

Construct	Medium	*lacZ* activity
pFAS3-Sst	- Inositol	6.0
	- Inositol + choline	5.8
	- Inositol + serine	6.5
	- Inositol + ethanolamine	5.5
	+ Inositol	2.6
	+ Inositol + choline	1.1
	+ Inositol + serine	2.8
	+ Inositol + ethanolamine	2.5
pFAS3-Sst-M1	- Inostol	1.4
Mutant of UAS_{INO}	+ Inositol	1.6
	+ Inositol + choline	1.8
pFAS3-840	- Inositol	3.5
	+ Inositol	2.0
	+ inositol + choline	1.2
pFAS3-840-M1	- Inositol	1.4
Mutant of UAS_{INO}	+ Inositol	1.5
	+ Inositol + choline	1.3
pFAS3-M2	- Inositol	4.5
	+ Inositol	2.1
	+ Inositol + choline	1.2
pFAS3-M1-M2	- Inositol	1.2
	+ Inositol	0.9
	+ Inositol + choline	1.0
CYC1-lacZ	- Inositol	0.3
	+ Inositol	0.3
	+ Inositol + choline	0.3
UAS_{INO}-*CYC1-lacZ*	- Inositol	40.0
	+ Inositol	21.0
	+ Inositol + choline	12.0

All the plasmids were tested in SEY2102. The inositol concentration was 200 mM and that of choline 2 mM. The concentrations of serine and ethanolamine were 200 μM.

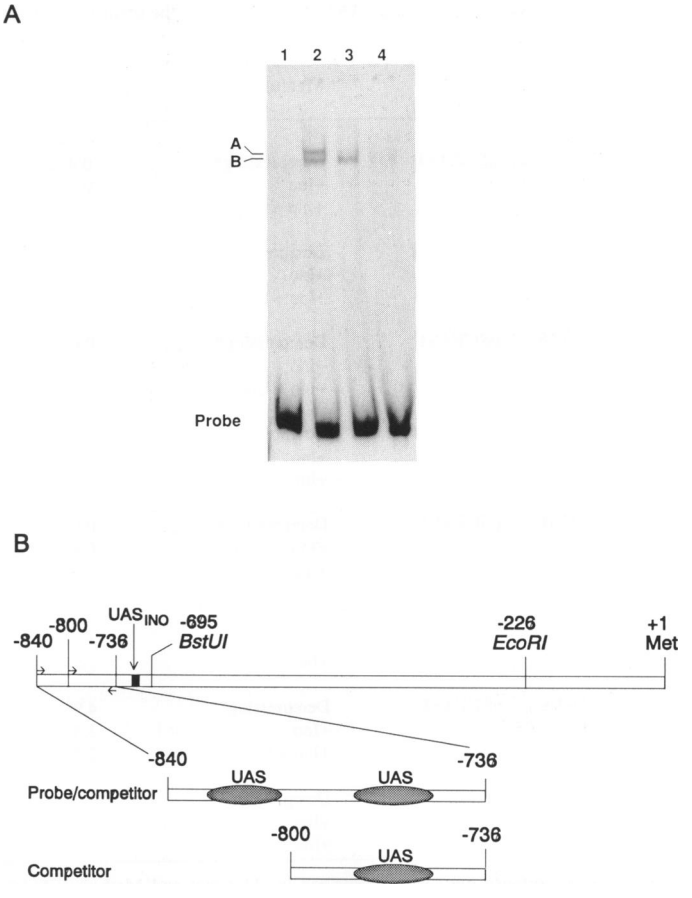

Figure 3. Electrophoretic mobility shift assay of the −840 to −736 region of *FAS3*. A 20-bases long forward primer starting from −840 and a reverse primer starting from but complimentary to the sequence at −736 (Figure 1) were used to amplify a 104 bp DNA fragment by using PCR. The nonlabeled DNA fragment (−800 to −736) was generated by using PCR as described above. The −840 oligonucleotide was end labeled using γ-[^{32}P]-ATP and polynucleotide kinase (17) to obtain a radioactive PCR product which was used as probe DNA. The extracts were made from BJ5465 were fractionated using ammonium sulfate. The 20 to 40% ammonium sulfate pellet fraction was dissolved in binding buffer, and the binding reactions were carried out as described (11, 25). The radioactive DNA was at approximately 1 ng, and the competitor DNA was in 1000 fold excess of the radioactive DNA. The reaction mixtures were analyzed on 4% acrylamide (29:1) in 0.5×TBE (11, 25). In (**A**), Lane 1, probe DNA (−840 to −736) in binding buffer; lane 2, protein complexes formed; lane 3, same as in lane 2 except that −800 to −736 DNA was used as a nonlabeled competitor; lane, 4, the −840 to −736 DNA was used as a nonlabeled competitor. The location of these DNA segments is described in Fig. 3B. In (**B**), the top line shows the functional regulatory regions identified through deletion and mutational analysis of *FAS3*. The arrows indicate the primers used in PCR mediated amplification of the specific DNA segments. The middle line shows the regulatory region consisting of two UAS elements. This DNA was used as radioactive probe as well as cold competitor in Fig. 3A. The last line shows the DNA segment containing only one UAS element which was used as cold competitor in Fig. 3A. The exact location of these two UAS elements has not been determined.

Further confirmation of the UAS function of the region between −840 to −744

To determine whether the region between −840 and −744 acts as UAS, the DNA segments −840 to −736 and −800 to −736 were cloned in a heterologous, UAS-lacking *CYC1-lacZ* reporter plasmid. As shown in Table 1, these DNA segments promoted the expression of the heterologous reporter gene. suggesting that there may be two UAS elements, one between −840 and −800 and the other between −800 and −736. These sequences were also found to be responsible for fatty acid-mediated repression (Table 1 and Figure 2). However, such repression was not observed with any of the reporter gene constructs containing only UAS_{INO} and GRF1 (Table 1). The presence of these UAS elements explains the observation that when UAS_{INO} is mutated fatty acid-mediated repression still occurs (Figure 2B). We have

Table 3. Expression of FAS1 and FAS3 reproter genes in the regulatory mutants of phospholipid biosynthesis

Strain	Plasmid	Medium	*lacZ* activity
it8	pSCFAS1	Derepressing*	0.4
ino2		+Ino	0.6
		+Ino + Cho	0.9
it8	pFAS3-Sst	Derepressing*	1.2
ino2		+Ino	1.1
		+Ino + Cho	1.3
SAH1028	pSCFAS1	Derepressing*	0.6
ino4		+Ino	1.2
		+Ino + Cho	1.4
SAH1028	pFAS3-Sst	Derepressing*	2.0
ino4		+Ino	2.5
		+Ino + Cho	1.8
SAH1032	pSCFAS1	Derepressing*	0.5
ino2,ino4		+Ino	0.9
		+Ino + Cho	2.0
SAH1032	pFAS3-Sst	Derepressing*	0.9
ino2,ino4		+Ino	1.1
		+Ino + Cho	1.2
W303-1A	pSCFAS1	Derepressing*	4.9
		+Ino	3.5
		+Ino + Cho	2.8
W303-1A	pFAS3-Sst	Derepressing*	6.5
		+Ino	3.0
		+Ino + Cho	2.0

The genotype of these mutants is described in 'Material and Methods'. Since these mutants do not grow in the absence of inositol, we have used 3 μM each of inositol and choline (the derepressing condition indicated by *), as recommended by Schuller *et al.* (29). For the repressing conditions 200 μM inositol 2 mM and choline were used. The mutant and the isogenic wild-type strains were transformed with pSCFAS1 reporter gene (11) and pFAS3-Sst (Figure 2) and the *lacZ* activity was determined.

performed electrophoretic mobility shift assays to test whether there are any factors in yeast cell extracts that bind to sequences in the region between −840 and −736. As shown in Figure 3, of the two complexes (A and B) that formed with −840 to −736 DNA, complex A, can be eliminated by competing with cold −800 to −736 DNA and both complexes by competing with −840 to −736 DNA. This suggests that both complexes are formed independently. Additional experiments are needed to identify the UAS sequences present in these regions and to determine their roles in fatty acid-mediated repression.

Functional UAS_{INO} is required for the phospholipid precursor-mediated repression of *FAS3*

We tested *FAS3-lacZ* reporter gene constructs that contained or lacked a functional UAS_{INO} for repression by soluble phospholipid precursors. As shown in Table 2, pFAS3-Sst, pFAS3-840, pFAS3-M2 and UAS_{INO}-*CYC1-lacZ* all showed inositol-mediated repression and the synergistic effect of inositol and choline on this repression. All these constructs contain a functional UAS_{INO}. The M1 mutants, the M1-M2 double mutants (Fig. 2) and *CYC1-lacZ*, all lacking UAS_{INO}, did not exhibit the inositol and choline-mediated repression. The inositol-mediated repression is at best 50% and the synergistic repression by inositol and choline is about 75% of that observed in the absence of inositol. As shown in Table 2, serine and ethanolamine

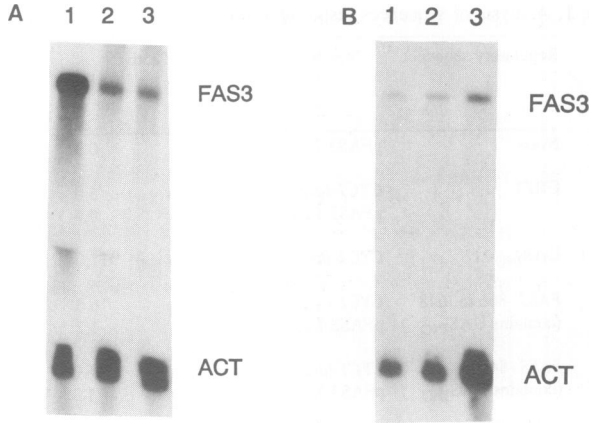

Figure 4. Northern analyses of *FAS3* mRNA levels. Total RNA was isolated from wild-type and *ino2-21* mutant grown under repressing and derepressing conditions. (A) RNA from wild-type cells (SEY2102) and (B) RNA from it8 (*ino2-21*) mutant were fractionated on a 1% agarose gel after glyoxal treatment (21). The RNA was transferred to nitrocellulose sheet and probed with *FAS3* and *ACT1* specific [^{32}P]-labeled nick translated probes (11, 17). (A) Lane 1, RNA from cells grown in the absence of inositol and choline (derepressing conditions); (B), lane 1, RNA from *ino2-21* mutant grown in the presence of inositol and choline but starved for inositol and choline for 6 hr. In both (A) and (B): lane 2, RNA from cells grown in the presence of 200 μM inositol; lane 3 RNA from cells grown in the presence of 200 μM inositol and 1mM choline. The autoradiograms were scanned densitometrically and the values normalized using actin. Considering the normalized densitometric values of *FAS3* mRNA in cells grown in the presence of inositol and choline (the values of the most repressing conditions [Lane 3]) as 1, *FAS3* mRNA levels in (A) 3.6 in lane 1, and 1.2 in lane 2. Similarly in (B), 0.9 in lane 1, and 0.9 in lane 2.

both in the presence and absence of inositol had no significant effect on the expression of the *FAS3-lacZ* reporter gene. We have previously shown that both FAS and ACC activities are reduced by about 50% and 75%, respectively, in the presence of inositol (11). However, when inositol (1mM) and choline (2 mM) were added to the assay mixture, they did not effect the activities of these enzymes (data not shown). The repression levels of *FAS3-lacZ* reporter gene were also observed at the level of *FAS3* mRNA (see below).

Regulatory genes of *INO1* also regulate the *FAS3-lacZ* reporter gene

There are three regulatory mutants that affect the expression of the *INO1* gene (13−16). *ino2* and *ino4* are considered to be mutants of the positive regulators of the genes involved in phospholipid biosynthesis. The regulatory mutant *opi* is a mutant of a negative regulator. Recently, it was shown that the UAS_{INO} is responsible for inositol and choline-mediated regulation (11, 12, 16). We have tested the expression of *FAS3-lacZ* reporter gene constructs in *ino2* and *ino4* regulatory mutant strains (Table 3). We used *FAS1-lacZ* for comparison. There are two functional UAS_{INO} sequences in the *FAS1* regulatory region that are critical for the expression of the gene (11, 12). The expression of the *FAS1-lacZ* and the *FAS3-lacZ* reporter genes in the *ino2*, and *ino4*, and the *ino2*, *ino4* double mutants was reduced severely. In addition, inositol and choline had no repressive effect on the reporter genes in these mutants. These results are consistent with the effects of these mutations on the genes involved in phospholipid synthesis (11−16). We also analyzed the levels of *FAS3* mRNA in *INO2* mutant and wild-type cells (Figure 4).

When the cells were grown in the absence of inositol and choline, the *FAS3* mRNA levels were about three times greater than the levels seen when the cells were grown in the presence of 200 mM inositol. This difference in the levels of *FAS3* mRNA is consistent with the three-fold difference in the ACC activity levels reported previously (Table 1 in Ref. 11). However, when the cells are exposed to derepressing conditions the *FAS3* mRNA levels did not increase in *INO2* mutant. These results are consistent with those obtained with reporter gene expression in these mutants as shown in Table 3, and with those reported by Schuller *et al.* (29) and Hablacher *et al.* (30).

DISCUSSION

We recently showed that all the three genes involved in fatty acid biosynthesis are repressed by fatty acids and are coordinately regulated (11). It has also been shown that *FAS1* and *FAS2* are regulated by inositol (11, 12). We demonstrated previously that the specific activity of ACC is reduced by 75% when the cells are grown in the presence of inositol (11). Consistent with these observations, the sequence of the *FAS3* regulatory region has a UAS_{INO}. Here we have shown that this UAS is functional based on deletion and mutational analyses. The Inositol and choline-mediated repression of the *lacZ* reporter genes, the increased levels of *FAS3* mRNA in the absence of inositol and choline, and the lack of repression of the reporter gene in the regulatory mutants of the *INO1*, all support our conclusion that *FAS3* gene is regulated by phospholipid precursors. These results further substantiate the idea that saturated fatty acid biosynthesis and phospholipid biosynthesis are coordinately regulated by the activator sequence UAS_{INO}. Consistent with this view is the finding that the gene coding for acyl-CoA binding protein (*ACB*) also has this UAS (31). Schuller *et al.* (29) have shown that the regulatory gene *INO4* regulates *FAS1* and *FAS2* and is autoregulated by UAS_{INO}. Very recently Hablacher *et al.* (30) suggested that *FAS3* is regulated by inositol and choline by measuring the mRNA levels in the regulatory mutants of phospholipid biosynthesis.

Kodaki *et al.* (30) have shown by mutational analysis that the core sequence of UAS_{INO} is TTCACATG. The mutational analyses of *FAS1* and *FAS2* reported previously (11) and the data shown in Figures 1 and 2, confirm that the core sequence CACATG is incomplete. Comparing the functional UAS_{INO} sequences in *FAS1*, *FAS2* and *FAS3* suggested that the minimal sequence of this UAS is TTCACATG ([11] and Figures 1 and 2B). The genes containing UAS_{INO} are repressed by phospholipid precursors to varying degrees (11–16). This variation is probably due to other regulatory sequences present in these genes ([11–16] and Figure 2, and Table 2), which would explain why yeast does not require fatty acids when grown in the presence of inositol and choline. Generally we observed that yeast cells grow better when inositol and choline are present in the culture medium. This influence of inositol and choline on growth may be due to the sparing effect these precursors create when present in the medium in the energy required to synthesize these compounds. The 50% to 75% reduction in the activity levels of FAS and ACC in the presence of the phospholipid precursors (11), the reporter gene expression, and the levels of FAS and ACC mRNAs (11,12, 29, 30 and Figure 4) suggest that the levels of these enzymes are in excess of what the cells need under laboratory conditions of growth. The presence of the UAS_{INO} in several genes involved in yeast lipid synthesis and metabolism suggests that this sequence modulates the expression of these genes so that an appropriate membrane lipid composition is maintained. Besides being a sequence that responds to inositol and choline, we have demonstrated through mutational analysis that, UAS_{INO} is needed as a positive regulator of *FAS* genes ([11] and the data presented herein). However, we are not sure whether *INO2* and *INO4* are the only positive regulators of *FAS* genes or additional genes are involved in this regulation. We have shown by deletion analysis of *FAS2*, that the UAS_{INO} by itself does not function efficiently as UAS, but mutating this sequence does cause 50% reduction in the expression of the gene (11). Consequently, it is possible that factors that bind UAS_{INO} may help recruit *trans*-acting factors that promote transcription, on the other UAS sequences present in *FAS* genes . We do not yet know the interactions between UAS_{INO} and the other regulatory sequences present in *FAS* genes and the regulatory factors that might influence these interactions.

The fatty acid-mediated repression of *FAS3-lacZ* reporter gene constructs is consistent with previous observations by Numa and coworkers (9, 10). Deletion analyses of *FAS1* and *FAS2* (11), and *FAS3* regulatory region (presented herein) did not identify a common sequence that would mediate this repression. However, the *FAS3* sequence between −840 and −736 might play a role in fatty acid mediated repression. We currently are investigating the role of these sequences in fatty acid-mediated repression.

ACKNOWLEDGEMENTS

This work was supported by grant DK-41872 from the National Institutes of Health. We are grateful to Dr S.J.Wakil for his encouragement and helpful discussions. We are thank Drs S.Henry for providing us the regulatory mutants, D.J.Stillman for computer analysis of the regulatory region, and W.-Y.Huang for making the oligonucleotides. We also thank M.Hsu, Dr M.H.Tai and Dr L.Abu-Al-Heiga for their help and valuable discussions and Ms P.P.Powell for her editorial review.

REFERENCES

1. Wakil,S.J., Stoops,J.K. and Joshi,V.C. (1983) *Annu. Rev. Biochem.* 52, 537–579.
2. Kuziora,M.A., Chalmers,J.H.Jr., Douglas,M.G., Hitzeman,R.A., Mattick,J.S. and Wakil,S.J. (1983) *J. Biol. Chem.* 258, 11648–11653.
3. Schweizer,M., Lebert,C., Holtke,J., Roberts,L.M. and Schweizer,E. (1984) *Mol.Gen. Gene.* 194, 457–465.
4. Chirala,S.S., Kuziora,M.A., Spector,D.M. and Wakil,S.J. (1987) *J. Biol. Chem.* 262, 4231–4240.
5. Schweizer,M., Roberts,L.M., Holtke,H.J., Takabayashi,K., Hollerer,E., Hoffmann,B., Muller,G., Kottig,H. and Schweizer,E. (1986) *Mol. Gen. Genet.* 203, 479–486.
6. Mohamed,A.H., Chirala,S.S., Mody,N.H., Huang,W.-Y. and Wakil,S.J. (1988) *J. Biol. Chem.* 263, 12315–12325.
7. Schweizer,E., Muller,G., Roberts,L.M., Schweizer,M., Rosch,J., Weisner,P., Beck,J., Startmann,D. and Zauner,I. (1987) *Fat. Sci. Technol.* 89, 570–577.
8. Al-Feel,W., Chirala,S.S. and Wakil,S.J. (1992) *Proc. Natl. Acad. Sci. USA* 89, 4534–4538.
9. Kamiryo,T., Parthasarathy,S. and Numa,S. (1976) *Proc. Natl. Acad. Sci. USA* 73, 386–390.
10. Numa,S. and Tanabe,T. (1984) in Numa,S. (ed.), Fatty Acid Metabolism and Its Regulation, Elsevier, Amsterdam pp. 1–27.
11. Chirala,S.S. (1992) *Proc. Natl. Acad. Sci. USA* 89, 10232–10236.
12. Schuller,H.-J., Hahn,A., Troster,F., Schutz,A. and Schweizer,E. (1992) *EMBO J.* 11, 107–114.
13. Carman,G.M. and Henry,S.A. (1989) *Annu. Rev. Biochem.* 58, 635–669.
14. Nikoloff,D.M. and Henry,S.A. (1991) *Annu. Rev. Genet.* 25, 559–583.

15. Lopes,J.M. and Henry,S.A. (1991) *Nucleic Acids Res.* 19, 3987–3994.
16. Bailis,A.M., Lopes,J.M., Kohlwein,S.D. and Henry,S.A. (1992) *Nucleic Acids Res.* 20, 1411–1418.
17. Maniatis,T., Fritsch,E.F. and Sambrook,J. (eds) (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor University Press. Cold Spring Harbor.
18. Chirala,S.S., Kasturi,R., Pazirandeh,M., Stolow,D.T., Huang,W.-Y. and Wakil,S.J. (1989) *J. Biol. Chem.* 264, 3750–3757.
19. Hahn,S., Pinkham,J., Wei,R., Miller,R. and Guarente,L. (1988) *Mol. Cell. Biol.* 8, 655–663.
20. Guarente,L., Yocum,R. and Gifford,P. (1982) *Proc. Natl. Acad. Sci. USA* 79, 7410–7414.
21. Slater,M.R. and Craig, E.A. (1987) *Mol. Cell.Biol.* 7, 1906–1916.
22. Ito,H., Fukuda,Y., Murata,K. and Kimura,A. (1983) *J. Bacteriol.* 153, 163–168.
23. Thomas,P.S. (1980) *Proc. Natl. Acad. Sci.USA* 77, 5201–5205
24. Pazirandeh,M., Chirala,S.S. and Wakil,S.J. (1991) *J. Biol. Chem.* 266, 20946–20952.
25. Buchman,A., Kimmerley,W.J., Rine,J. and Kornberg,R.D. (1988) *Mol. Cell.Biol.* 8, 210–225.
26. Verdier,J.-M. (1990) *Yeast* 6, 271–297.
27. Chasman,D.I., Leu,N.F., Buchman,A.R., LaPointe,J.W., Lorch,Y. and Kornberg,R.D. (1990) *Genes Dev.* 4, 503–514.
28. Buchman,A.R., Lue,N.F. and Kornberg,R.D. (1988) *Mol. Cell. Biol.* 8, 5086–5099.
29. Schuller,H.-J., Schorr,R. and Schweizer,E. (1992) *Nucleic Acids Res.* 20, 5955–5961.
30. Hablacher,M., Ivessa,A.S., Paltauf,F. and Kohlwein,S.D. (1993) *J. Biol. Chem.* 268,10946–10952.
31. Rose,T.M., Schultz,E.R. and Todaro,G.J. (1992) *Proc. Natl. Acad. Sci. USA* 89, 11287–11291.
30. Kodaki,T., Nikawa,J-I., Hosaka,K. and Yamashita,S. (1991) *J. Bacteriol.* 173, 7992–7995.